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EVALUATION THE EXPRESSION OF THREE GENES TO EPITHELIAL OVARIAN CANCER RISK IN CHINESE POPULATION

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Abstract

Background: Ovarian cancer is associated with poor survival, because patients are diagnosed at an advanced stage of the disease, and in addition, tumors develop chemoresistance, which carries a poor prognosis for the patient.

Material and methods: We hypothesize that high expression of SDF-1, survivin and smac is associated with ovarian cancers development and could be used as a biomarker to identify this disease. The expressions of SDF-1, survivin and smac in normal ovarian (NO) tissue, benign tumor (BT) tissue and epithelial ovarian cancer (EOC) tissue were immunohistochemically analysed.

Results: Positive expressions of SDF-1, survivin and smac were significantly higher in EOC tissue than those in NO and BT tissues. SDF-1 expressions were significantly more weaker in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and SDF-1 positivity ($P < 0.05$). Survivin expressions were significantly more stronger in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and surviving positivity ($P < 0.05$). Smac expressions were significantly more stronger in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and smac positivity ($P < 0.05$).

Conclusion: These results indicate that SDF-1, surviving and smac are closely associated with EOC metastasis.

Key words: SDF-1, Expression, Gene, Ovarian cancers

Introduction

Ovarian cancer is associated with poor survival, because patients are diagnosed at an advanced stage of the disease, and in addition, tumors develop chemoresistance,which carries a poor prognosis for the patient (Eltabbakh & Awtrey, 2001). Because of its insidious growth, it commonly remains asymptomatic for long periods of time and finally presents with signs and symptoms related to the presence of locally advanced or even metastatic disease. As a consequence, the majority of these patients are diagnosed when treatment options are limited, and the overall 5-year survival rates do not exceed 30% (FIGO (International Federation of Gynecology and Obstetrics), 2003; Richardon et al, 1985).

Stromal-derived factor-1 (SDF1; also called CXCL12), a chemokine originally isolated from bone marrow stromal cells, is constitutively expressed in several tissues (Sun et al., 2011; Teicher and Fricker, 2010). SDF1 is the only known natural ligand of chemokine CXC motif receptor 4 (CXCR4), which is expressed by numerous cell types, including leukocytes, and endothelial, epithelial, smooth muscle and cancer cells (Sun et al., 2011; Teicher and Fricker, 2010). SDF-1 exerts its activity by interacting with the CXCR4 receptor and leads to cancer cell migration, invasion and proliferation (Schrader et al, 2002).

Survivin is a newly discovered member of the inhibitors of the apoptosis (IAP) family, with a potential involvement in malignant transformation and tumor growth. Survivin is expressed during fetal development but not in normal adult tissues

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(Ambrosini et al, 1997). High expression levels of this antiapoptotic protein have been previously found in epithelial carcinomas located in the gastrointestinal tract (Kawasaki et al, 1998; Sarela et al, 2000), breast (Tanaka et al, 2000), lung (Monzo et al, 1999), and ovary (Sui et al, 2002; Takai et al, 2002), and associated with increased biological aggressiveness, elevated proliferative activity, and several unfavorable prognostic parameters, such as increased stage or reduced survival.

Smac, also known as direct inhibitor of apoptosis binding protein with low isoelectric point (DIABLO), is a mithochondrial protein containing an NHterminal SS-amino-acid mitochondrial import sequence, released from mitochondria into the cytosol in response to apoptotic stimuli (Du et al, 2000; Verhagen et al, 2000). Once released into the cytosol, Smac interacts with IAPs at the level of the BIR domains via an NH2-terminal motif, thereby eliminating the inhibitory effects of IAPs on caspase-3, caspase-7 and caspase-9 (Verhagen et al, 2000; Sun et al, 2016). The interaction of Smac with IAPs results in a rapid ubiquitination and subsequent degradation of released Smac, mediated by the ubiquitin–protein ligase function of some IAPs (MacFarlane et al, 2002). Mitochondrial Smac release is suppressed by Bcl-2 and Bcl-XL (Vyas et al, 2004).

In this study, we investigate whether the expression of SDF-1, survivin, and smac correlate with clinical characteristics and cancer-specific survival of patients.

Materials and methods

Samples

A total of 125 paraffin-embedded tissue samples were collected from the archives of the Department of Pathology, Shantou University, between February 2013 and October 2014. The tissue samples consist of 60 EOC tissues, 40 benign tumor tissues (BT) and 25 normal ovaries tissues (NO) (Table 1). Fresh surgical specimens were collected between May 2013 and December 2014, snap-frozen in liquid nitrogen immediately after resection, and were stored at −80 °C for immunohistochemistry analysis. The clinical and pathological characteristics of these patients are described in Tables 1 and 2. The patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO). All tissue blocks were reviewed for histological type and graded by two senior pathologists, stained with hematoxylin–eosin. The study protocols have been approved by the local Ethics Committee. Prior informed consent was obtained from the patients according to the Declaration of Helsinki.

Table 1: SDF-1, survivin and smac positive expression in ovarian tissues

Group	n	SDF-1 positive expression				survivin positive expression				smac positive expression			
		-	+	++	+++	-	+	++	+++	-	+	++	+++
NO tissues	25	24	1	0	0	25	0	0	0	0	6	12	7
BT tissues	40	33	6	1	0	28	6	4	2	1	13	10	16
EOC tissues	60	0	32	15	13	0	24	16	20	0	28	15	17

Immunohistochemistry

Sections of formalin-embedded tissues (4 μm) were deparaffinized in xylene and washed in graded ethanol. For antigen retrieval, samples were immersed in Tris–EDTA buffer and incubated at 125 °C for 2 min 30 s. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min. Samples were incubated with primary antibody for 1 h at room temperature. The following primary antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA): rabbit monoclonal antibody SDF-1 (1:25 dilution), rabbit monoclonal antibody survivin (1:100 dilution) and mouse monoclonal antibody smac (1:100 dilution). Samples were treated with Envision-plus reagent (Dako, Glostrup, Denmark) for 30 min and diaminobenzidine as a chromogen for 5 min followed by counterstaining with hematoxylin. Prostate adenocarcinoma tissues served as the positive control.

Staining was evaluated by a specialized pathologist who was unaware of the patient's clinical features. For quantitative analysis,

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both the percentage of stained cells and intensity were assessed in 10 high-power fields, as described previously (Rojo et al, 2007). If tumor cells were stained more than 1%, the case was considered as the positive. For the analysis of the correlation between biomarkers, immunohistochemical score was calculated by multiplying the intensity score (0 to 3) and fraction score (0 to 100). Immunohistochemical findings were correlated with clinicopathological characteristics such as stage, histologic grade, lymph node metastasis, and ascites.

Statistical methods

Statistical analyses were performed using the SPSS 15.0 statistical software package. Correlations between SDF-1, surviving and smac expression and the clinicopathologic parameters were evaluated by χ^2 test. Correlations between various factors were assessed by Spearman rank correlations. The criterion for inclusion of a variable was $p < 0.05$ in the univariate analysis and for removing a variable $p > 0.05$ in the multivariate analysis.

Results

We performed immunohistochemical staining to analyze the expression of SDF-1, survivin and smac in 25 normal ovaries, 40 benign tumors, and 60 EOC. Representative samples of SDF-1, survivin and smac staining are displayed in Table 1. SDF-1, survivin and smac positive staining predominantly showed cytoplasmic and membrane localization, and EOC specimens were strongly positive for SDF-1, and survivin expression. In contrast, SDF-1, and survivin immunoreactivity was negatively or weakly observed in the normal and benign tumors.

Differences in proportions were evaluated by χ^2 or Fisher's test, whichever is appropriate. The SPSS statistical software system for Windows (Statistical Package for Social Sciences, version 10.0, Chicago, IL, USA) was used for the statistical analysis. $P < 0.05$ was considered to be statistically significant.

As indicated in Table 2, SDF-1 expressions were significantly more weaker in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. FIGO stage and histological grade were not associated with SDF-1 staining ($P > 0.05$). In contrast, SDF-1 expressions were significantly stronger in EOC patients with lymph node metastasis and with ascites. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and SDF-1 positivity ($P < 0.05$).

Table 2: Clinicopathologic parameters in 60 patients with EOC according to SDF-1, survivin and smac positive expression

clinicopathological characteristics	n	SDF-1 positive expression		χ^2	p	survivin positive expression		χ^2	p	smac positive expression		χ^2	p
		+	++ ～ +++			+	++ ～ +++			+	++ ～ +++		
histological grade													
Middle, low	39	20	20	0.536	0.464	2	20	12.609	0.000	18	5	14.958	0.000
High	21	12	8			22	18			10	27		
FIGO stage													
I-II	37	23	17	0.837	0.360	18	6	20.417	0.000	16	6	9.479	0.002
III-IV	23	9	11			6	30			12	26		
lymph node metastasis													
Yes	45	16	22	5.249	0.022	15	31	4.488	0.034	16	28	7.037	0.008
No	15	16	6			9	5			12	4		

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Ascites													
Yes	38	19	24	5.102	0.02	10	33	17.7	0.00	19	29	4.83	0.02
No	22	13	4		4	14	3	29	0	9	3	8	8

Survivin expressions were significantly more stronger in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. FIGO stage and histological grade were positively associated with surviving positivity ($P < 0.05$). In addition, survivin expressions were significantly stronger in EOC patients with lymph node metastasis and with ascites. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and surviving positivity ($P < 0.05$, Table 2).

Smac expressions were significantly more stronger in advanced ovarian carcinomas (FIGO stage III–IV), and in high-grade carcinomas. FIGO stage and histological grade were positively associated with smac positivity ($P < 0.05$). In addition, smac expressions were significantly stronger in EOC patients with lymph node metastasis and with ascites. There was a positive correlation between EOC patients with lymph node metastasis and with ascites and smac positivity ($P < 0.05$, Table 2).

Discussion

The purpose of the present study was to investigate the prognostic significance of SDF-1, survivin and smac expression in EOC patients, and to correlate the pattern of expression with clinicopathological parameters.

SDF-1 has been shown to be expressed constitutively by a wide variety of tissues (Tashiro et al, 1993; Shirozu et al, 1995; Yang et al, 2015). Moreover, Arai et al. have shown that stromal cells, not lymphocytes, in lymph nodes expressed SDF-1 and attracted B-lymphoma cells (Arai et al, 2000). In different carcinomas increased SDF-1 expression is associated with metastasis and poor survival or with improved survival (Lee et al, 2012; Schrevel et al, 2012; Zhao et al, 2011; Razis et al, 2012; Kurtuncu et al, 2016). There may be such contradictory observations because in these studies SDF-1 expression was detected by immunohistochemistry and, thus, they did not distinguish which SDF-1 isoform was responsible for the observed higher SDF-1 protein expression. SDF-1 expression in cancer is also epigenetically regulated by promoter methylation and SDF-1 gene polymorphism probably increases SDF-1 expression (Zhi et al, 2012; de Oliveira et al, 2011). Our current study shows that SDF-1 positive expression in EOC patients was significantly stronger than one in patients with benign tumors and healthy people.

Survivin expression in cancer has been the subject of intense research due to its widely recognized anti-apoptotic properties and attractive potential as a therapeutic target. This anti-apoptotic gene is predominantly expressed in cancer and considered to be a prognostic factor for various cancers (Sarela et al, 2000; Islam et al, 2000). Survivin expression in diverse tumor tissues has been linked with shorter overall and disease-free survival (Ibrahim et al, 2012; Huang et al, 2011; Antonacopoulou et al, 2010; Wagner et al, 2006; Span et al, 2006). The subcellular location of Survivin has been shown to have an important influence on tumor biology. Survivin has been found in the mitochondria of cancer cells (Dohi et al, 2004; Ling et al, 2007), but not of normal tissues suggesting that this subcellular compartmentalization has a role in tumor pathogenesis (Jha et al, 2012). The subcellular distribution of survivin seems to be altered during growth and progression of ovarian carcinoma under the influence of yet unknown molecular mechanisms. However, the exact prognostic and clinical implications of the immunohistochemical localization (nuclear or cytoplasmic) of survivin in ovarian carcinoma remain controversial. Tringler et al. (2004) found that nuclear localization of survivin was more common in benign or borderline than in malignant serous tumors, and more intense in low-grade versus high-grade malignant tumors. In contrast to these findings, Yoshida et al. (2001) gave evidence that the disease-free interval in ovarian carcinoma patients was significantly shorter when survivin expression was observed in the nucleus compared to the cytoplasm. In our study, we found that survivin positive expression in EOC patients was significantly stronger than one in patients with benign tumors and healthy people.

Smac (also known as DIABLO: direct IAP binding protein with low PI), was initially discovered independently by two groups in 2000 (Du et al, 2000; Verhagen et al, 2000). The human gene is located on chromosome 12p and is composed of seven exons. The 1.5 kb cDNA of Smac encodes 239 amino acids, producing a protein of 27 kDa. The first 55 amino acids constitute a mitochondrial targeting signal peptide and are cleaved after import into the mitochondria to generate mature Smac. Because Smac levels could

determine the sensitivity of cancer cells to apoptosis, the expression levels of Smac could be useful as a prognostic or therapeutic marker. Tumor cells usually exhibit low Smac levels and resistance to apoptosis, so Smac expression is inversely correlated with sensitivity to apoptosis. Previous studies based on the enforced expression of Smac/DIABLO in tumor cells using retroviral vectors have provided variable results showing either caspase activation and in some ovarian cancer cell lines induction of apoptosis (McNeish et al, 2003; McNeish et al, 2005) or no effect on caspase activation and no induction of apoptosis in neuroblastoma cells (Fulda et al, 2002). Furthermore, Smac peptides added alone failed to induce apoptosis of neuroblastoma (Fulda et al, 2002), glioblastoma (Fulda et al, 2002; Mizukawa et al, 2006) and leukemic (Guo et al, 2002) cells, but greatly sensitize these cells to the proapoptotic effects of TRAIL. Our current study shows that smac positive expression in EOC patients was significantly stronger than one in patients with benign tumors and healthy people.

In our current study, SDF-1, surviving and smac expressions are strong in EOC patients, indicating that SDF-1, surviving and smac are closely associated with EOC development. In addition, statistical analysis shows that FIGO stage and histological grade were not associated with SDF-1 staining ($P > 0.05$). However, FIGO stage and histological grade were positively associated with surviving and smac staining ($P > 0.05$). There was a positive correlation between EOC patients with lymph node metastasis and with ascites and SDF-1, surviving or smac positivity ($P < 0.05$). These results indicate that surviving and smac are correlated with clinical stage and lymph node metastasis of EOC patients.

References

1. Ambrosini, G., Adida, C., Altieri, D.C. (1997). A novel antiapoptosis gene, survivin, expressed in cancer and lymphoma, Nat. Med. 3: 917–921.
2. Antonacopoulou, A.G., Floratou, K., Bravou, V., Kottorou, A. (2010). F.I. Dimitrakopoulos, S. Marousi, M. Stavropoulos, A.K. Koutras, C.D. Scopa, H.P. Kalofonos, The survivin -31 snp in human colorectal cancer correlates with survivin splice variant expression and improved overall survival, Anal. Cell Pathol. (Amsterdam) 33: 177–189.
3. Arai, J., Yasukawa, M., Yakushijin, Y., Miyazaki, T., Fujita, S. (2000). Stromal cells in lymph nodes attract B-lymphoma cells via production of stromal cell-derived factor-1, Eur. J. Haematol. 64: 323–332.
4. de Oliveira, K.B., Guembarovski, R.L., Oda, J.M., Mantovani, M.S., Carrera, C.M., Reiche, E.M., Voltarelli, J.C., da Silva do Amaral Herrera, A.C., Watanabe, M.A. (2011). CXCL12 rs1801157 polymorphism and expression in peripheral blood from breast cancer patients. Cytokine, 55: 260.
5. Dohi, T., Beltrami, E., Wall, N.R., Plescia, J., Altieri, D.C. (2004). Mitochondrial surviving inhibits apoptosis and promotes tumorigenesis, J. Clin. Investig. 114: 1117–1127.
6. Du, C., Fang, M., Li, Y., Li, L., Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell. 102: 33–42.
7. Du, C.Y., Fang, M., Li, Y.C., Li, L., Wang, X.D. (2000). Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition, Cell 102: 33–42.
8. Eltabbakh, G.H., Awtrey, C.S. (2001). Current treatment for ovarian cancer. Expert Opin Pharmacother. 2:, 109–124.
9. FIGO (International Federation of Gynecology and Obstetrics), Annual report on the results of treatment in gynecological cancer, Int. J. Gynaecol. Obstet. 83 (Suppl. 1) (2003) 1–229.
10. Fulda, S., Wick, W., Weller, M., Debatin, K.M. (2002). Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med. 8: 808–815.
11. Guo, F., Nimmanapalli, R., Paranawithana, S., Wittman, S., Griffin, D., Bali, P., O'Bryan, E., Fumero, C., Wang, H.G., Bhalla, K. (2002). Ectopic overexpression of second mitochondria-derived activator of caspases (Smac/DIABLO) or cotreatment with N-terminus of Smac/DIABLO peptide potentiates epothilone B derivative-(BMS 247550) and Apo-2L/TRAIL-induced apoptosis. Blood. 99: 3419–3426.
12. Huang, Y., Chen, X., Chen, N., Nie, L., Xu, M., Zhou, Q. (2011). Expression and prognostic significance of survivin splice variants in diffusely infiltrating astrocytoma, J. Clin. Pathol. 64: 953–959.

13. Ibrahim, A.M., Mansour, I.M., Wilson, M.M., Mokhtar, D.A., Helal, A.M., Al Wakeel, H.M. (2012). Study of survivin and X-linked inhibitor of apoptosis protein (XIAP) genes in acute myeloid leukemia (AML), Lab Hematol 18: 1–10.
14. Islam, A., Kageyama, H., Takada, N., Kawamoto, T., Takayasu, H., Isogai, E., Ohira, M., Hashizume, K., Kobayashi, H., Kaneko, Y., Nakagawara, A. (2000). High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma, Oncogene 19: 617±623.
15. Jha, K., Shukla, M., Pandey, M. (2012). Survivin expression and targeting in breast cancer, Surg. Oncol. 21: 125–131.
16. Kawasaki, H., Altieri, D.C., Lu, C.D., Toyoda, M., Tenjo, T., Tanigawa, N. (1998). Inhibition of apoptosis by survivin predicts shorter survival rates of colorectal cancer, Cancer Res. 58: 5071–5074.
17. Kurtuncu, M., Yildiz, H., Akhan, L.U. (2016). The Use of Complementary and Alternative Therapies in Childhood Cancer: A Questionnaire Based On A Descriptive Survey From The Western Black Sea Region Of Turkey. Afr J Tradit Complement Altern Med. 13(3):66-74
18. Lee, H.J., Lee, K., Lee, D.G., Bae, K.H., Kim, J.S., Liang, Z.L., Huang, S.M., Suk Oh, Y,, Kim, H.Y., Jo, D.Y., Min, J.K., Kim, J.M., Lee, H.J. (2012). Chemokine (C-X-C motif) ligand 12 is associated with gallbladder carcinoma progression and is a novel independent poor prognostic factor. Clin Cancer Res. 18: 3270.
19. Ling, X., Cheng, Q., Black, J.D., Li, F. (2007). Forced expression of survivin-2B abrogates mitotic cells and induces mitochondria-dependent apoptosis by blockade of tubulin polymerization and modulation of Bcl-2, Bax, and survivin, J. Biol. Chem. 282: 27204–27214.
20. MacFarlane, M., Merrison, W., Bratton, S.B., Cohen, G.M. (2002). Proteasomemediated degradation of Smac during apoptosis: XIAP promotes Smac ubiquitination in vitro. J Biol Chem, 277: 36611–36616.
21. McNeish, I.A., Bell, S., McKay, T., Tenev, T., Marani, M., Lemoine, N.R. (2003). Expression of Smac/DIABLO in ovarian carcinoma cells induces apoptosis via a caspase-9-mediated pathway. Exp Cell Res. 286: 186–198.
22. McNeish, I.A., Lopes, R., Bell, S.J., McKay, T.R., Fernandez, M., Lockley, M., Wheatley, S.P., Lemoine, N.R. (2005). Survivin interacts with Smac/DIABLO in ovarian carcinoma cells but is redundant in Smac-mediated apoptosis. Exp Cell Res. 302: 69–82.
23. Mizukawa, K., Kawamura, A., Sasayama, T., Tanaka, K., Kamei, M., Sasaki, M., Kohmura, E. (2006). Synthetic Smac peptide enhances the effect of etoposide-induced apoptosis in human glioblastoma cell lines. J Neuro-Oncol. 77: 247–255.
24. Monzo, M., Rosell, R., Felip, E., Astudillo, J., Sanchez, J.J., Maestre, J., Martin, C., Font, A., Barnadas, A., Abad, A. (1999). A novel anti-apoptosis gene: re-expression of surviving messenger RNA as a prognosis marker in non-small-cell lung cancers, J. Clin. Oncol. 17: 2100–2104.
25. Richardon, G.S., Scully, R.E., Nikrui, N., Nelson Jr., J.H. (1985). Common epithelial cancer of the ovary (2), N. Engl. J. Med. 312: 474–483.
26. Rojo, F., Najera, L., Lirola, J., Jiménez, J., Guzmán, M., Sabadell, M.D., Baselga, J., Ramon y Cajal, S. (2007). 4E-binding protein 1, a cell signaling hallmark in breast cancer that correlates with pathologic grade and prognosis. Clin Cancer Res. 13(1): 81–89.
27. Sarela, A.I., Macadam, R.C., Farmery, S.M., Markham, A.F., Guillou, P.J. (2000). Expression of the antiapoptosis gene, survivin, predicts death from recurrent colorectal carcinoma, Gut 46: 645–650.
28. Schrader, A., Lechner, O., Templin, M., Dittmar, K.E., Machtens, S., Mengel, M., Probst-Kepper, M., Franzke, A., Wollensak, T., Gatzlaff, P., Atzpodien, J., Buer, J., Lauber J. (2002). CXCR4/CXCL12 expression and signaling in kidney cancer. Br J Cancer. 86: 1250–1256.
29. Schrevel, M., Karim, R., ter Haar, N.T., van der Burg, S.H., Trimbos, J.B., Fleuren, G.J., Gorter, A., Jordanova, E.S. (2012). CXCR7 expression is associated with disease-free and disease-specific survival in cervical cancer patients. Br J Cancer; 106: 1520.
30. Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T., Honjo, T. (1995). Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene, Genomics 28: 495–500.
31. Span, P.N., Tjan-Heijnen, V.C., Heuvel, J.J., de Kok, J.B., Foekens, J.A., Sweep, F.C. (2006). Do the survivin (BIRC5) splice

variants modulate or add to the prognostic value of total survivin in breast cancer?, Clin Chem. 52: 1693–1700.

32. Sui, L., Dong, Y., Ohno, M., Watanabe, Y., Sugimoto, K., Tokuda, M. (2002). Survivin expression and its correlation with cell proliferation and prognosis in epithelial ovarian tumors, Int. J. Oncol. 21: 315–320.
33. Sun, M.-Y., Ye, Y., Xiao, L., Rahman, K., Xia, W., Zhang, H. (2016). DAIDZEIN: A REVIEW OF PHARMACOLOGICAL EFFECTS. Afr J Tradit Complement Altern Med. 13(3):117-132
34. Sun, X., Cheng, G., Hao, M., Zheng, J., Zhou, X., Zhang, J., Taichman, R.S., Pienta, K.J., Wang, J., (2011). CXCL12/CXCR4/CXCR7 chemokine axis and cancer progression. Cancer Metastasis Rev. 29: 709–722.
35. Takai, N., Miyazaki, T., Nishida, M., Nasu, K., Miyakawa, I. (2002). Expression of survivin is associated with malignant potential in epithelial ovarian carcinoma, Int. J. Mol. Med. 10: 211–216.
36. Tanaka, K., Iwamoto, S., Gon, G., Nohara, T., Iwamoto, M., Tanigawa, N. (2000). Expression of survivin and its relationship to loss of apoptosis in breast carcinomas, Clin. Cancer Res. 6: 127–134.
37. Tashiro, K., Tada, H., Heilker, R., Shirozu, M., Nakano, T., Honjo, T. (1993). Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins, Science 261: 600–603.
38. Teicher, B.A., Fricker, S.P., (2010). CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin. Cancer Res. 16: 2927–2932.
39. Tringler, B., Lehner, R., Shroyer, A.L., Shroyer, K.R. (2004). Immunohistochemical localization of survivin in serous tumors of the ovary, Appl. Immunohistochem. Mol. Morphol. 12: 40–43.
40. Verhagen, A.M., Ekert, P.G., Pakusch, M., Silke, J., Connolly, L.M., Reid, G.E., Moritz, R.L., Simpson, R.J., Vaux, D.L. (2000). Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell. 102: 43–53.
41. Vyas, S., Juin, P., Hancock, D., Suzuki, Y., Takahashi, R., Triller, A., Evan, G. (2004). Differentiation-dependent sensitivity to apoptogenic factors in PC12 cells. J Biol Chem; 279: 30983–30993.
42. Zhao, B.C., Wang, Z.J., Mao, W.Z. et al. (2011). CXCR4/SDF-1 axis is involved in lymph node metastasis of gastric carcinoma. World J Gastroenterol. 17: 2389.
43. Razis, E., Kalogeras, K.T., Kotoula, V., Eleftheraki, A.G., Nikitas, N., Kronenwett, R., Timotheadou, E., Christodoulou, C., Pectasides, D., Gogas, H., Wirtz, R.M., Makatsoris, T., Bafaloukos, D., Aravantinos, G., Televantou, D., Pavlidis, N., Fountzilas, G. (2012). Improved outcome of high-risk early HER2 positive breast cancer with high CXCL13-CXCR5 messenger RNA expression. Clin Breast Cancer. 12: 183.
44. Verhagen, A.M., Ekert, P.G., Pakusch, M., Silke, J., Connolly, L.M., Reid, G.E., Moritz, R.L., Simpson, R.J., Vaux, D.L. (2000). Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins, Cell 102: 43–53.
45. Wagner, M., Schmelz, K., Wuchter, C., Ludwig, W.D., Dorken, B., Tamm, I. (2006). In vivo expression of survivin and its splice variant survivin-2B: impact on clinical outcome in acute myeloid leukemia, Int. J. Cancer 119:, 1291–1297.
46. Yang, Y.H., Zhao, R.H., Hao, Z.P., Li, L., Xu, C., Cui, Y.Y. (2015). Effects Of Danchi Decoction On P450arom, Survivin Of Eutopic Endometrium Of Patients With Endometriosis After Conservative Surgery. Afr J Tradit Complement Altern Med. 12(4):65-71.
47. Yoshida, H., Ishiko, O., Sumi, T., Matsumoto, Y., Ogita, S. (2001). Survivin, bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas, Int. J. Oncol. 19: 537–542.
48. Zhi, Y., Chen, J., Zhang, S. et al. (2012). Down-regulation of CXCL12 by DNA hypermethylation and its involvement in gastric cancer metastatic progression. Dig Dis Sci. 57: 650.